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Tolerance of Bentgrass (*Agrostis*) Species and Cultivars to Methiozolin

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Methiozolin is a new herbicide from South Korea currently under development in the United States for PRE and POST annual bluegrass control in bentgrass and most other cool- and warm-season turfgrasses. Greenhouse studies were conducted in 2012 at the University of California, Riverside, CA, and Auburn University, Auburn, AL, to evaluate the relative tolerance of three bentgrass species comprised of nine creeping bentgrass (CRBG) cultivars, velvet bentgrass (VBG) and colonial bentgrass (COBG) to methiozolin at 0, 0.6, 1.1, 2.2, 4.5 and 9.0 kg ai ha^{-1}. Methiozolin was applied 7 wk after seeding, followed by a second application 5 wk later. Methiozolin rates that produced 25% injury (TI_{25}) and 50% clipping dry weight reduction (GR_{50}) relative to a nontreated control for each species or cultivar were calculated using four-parameter logistic regression. Turf injury rates at 21 d after second treatment (DAT2) were the most consistent in describing relative tolerance among bentgrass species. Overall, CRBG was more tolerant to methiozolin than VBG or COBG. After two applications, methiozolin rates that caused TI_{25} were 1.1, 0.2, and 0.3 kg ha^{-1} for CRBG (across all cultivars), VBG, and COBG, respectively. VBG and COBG were not tolerant of sequential methiozolin applications at rates necessary to control annual bluegrass under field conditions. Herbicide rates that caused TI_{25} and GR_{50} decreased with the second application. 'Penn A-4' CRBG exhibited the highest TI_{25} 28 d after initial treatment (DAIT) at University of California at Riverside (4.5 kg ha^{-1}), but only 2.5 kg ha^{-1} with two applications by 21 DAT2. All CRBG cultivars tested tolerated methiozolin at 0.5 kg ha^{-1}, the recommended sequential use rate for putting greens in Korea.

Nomenclature: Methiozolin, MRC-01, 5-(2,6-difluoro-benzyloxymethyl)-5-methyl-3-(3-methylthiophen-2-yl)-4,5-dihydro-isoxazole; annual bluegrass, *Poa annua* L.; creeping bentgrass, *Agrostis stolonifera* L.; colonial bentgrass, *Agrostis capillaris* L.; velvet bentgrass, *Agrostis canina* L.

Key words: Four-parameter logistic regression, growth reduction, herbicide sensitivity.

Methiozolin es un herbicida nuevo proveniente de Korea del Sur que está actualmente siendo desarrollado en los Estados Unidos para el control PRE y POST de *Poa annua* en *Agrostis* spp. (bentgrass) y la mayoría de otros céspedes de clima frío y cálido. Se realizaron estudios de invernadero en 2012 en la Universidad de California, Riverside, California y en la Universidad Auburn, Auburn, Alabama, para evaluar la tolerancia relativa de tres especies de *Agrostis* incluyendo nueve cultivares de *Agrostis stolonifera* (CRBG), *Agrostis canina* (VBG) y *Agrostis capillaris* (COBG) a methiozolin a 0, 0.6, 1.1, 2.2, 4.5, y 9.0 kg ai ha^{-1}. Methiozolin fue aplicado 7 semanas después de la siembra, seguido por una segunda aplicación 5 semanas después. Las dosis de methiozolin que produjeron 25% de daño (TI_{25}) y una reducción del 50% en el peso seco (GR_{50}) de los residuos de poda (clippings) en relación al testigo no-tratado para cada especie o cultivar fueron calculados usando una regresión logística de cuatro parámetros. El daño en el césped según la dosis a 21 d después del segundo tratamiento (DAT2) fue el más consistente al describir la tolerancia relativa entre especies de *Agrostis*. En general, CRBG fue más tolerante a methiozolin que VBG o COBG. Después de dos aplicaciones, las dosis de methiozolin que causaron TI_{25} fueron 1.1, 0.2, y 0.3 kg ha^{-1} para CRBG (promediado para todos los cultivares), VBG, y COBG, respectivamente. VBG y COBG no fueron tolerantes a aplicaciones secuenciales de methiozolin a dosis que son necesarias para el control de *Poa annua* bajo condiciones de campo. Las dosis de herbicida que causaron TI_{25} y GR_{50} disminuyeron con la segunda aplicación. CRBG 'Penn A-4' mostró la mayor TI_{25} 28 d después del tratamiento inicial (DAIT) en la Universidad de California en Riverside (4.5 kg ha^{-1}), pero solamente 2.5 kg ha^{-1} con dos aplicaciones a 21 DAT2. Todos los cultivares de CRBG evaluados toleraron methiozolin a 0.5 kg ha^{-1}, la cual es la dosis secuencial recomendada para el uso en putting greens en Korea.

Bentgrasses (BG) (*Agrostis* spp.) are utilized for intensively managed turf areas, including tees, fairways, and putting greens due to their superior color, density, and texture when maintained at a low height of cut (Beard 1973). The dominant species in the United States is creeping bentgrass

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(CRBG), but velvet bentgrass (VBG) and colonial bentgrass (COBG) are also used in climates where they are adapted. Annual bluegrass (AB) can be a desirable turfgrass species, but is often considered a weed in stands of newly established or highly maintained BG, and AB control is one of the biggest challenges faced by turfgrass managers (Beard 1973; Lycan and Hart 2005). Annual bluegrass suppression in BG putting greens is currently achieved with plant growth regulators and cultural practices aimed at giving the competitive advantage to BG (Flessner et al. 2013; Johnson and Murphy 1996). Although several herbicides are available in the United States for control of AB, none are labeled for POST use on BG putting greens, and only bensulide is labeled for PRE control (Anonymous 2009; Flessner et al. 2013).

Methiozolin (MRC-01; Moghu Research Center Ltd., BVC 311, KRIBB, Yuseong, Daejon, 305–333, South Korea) is a new turfgrass herbicide in the isoxazoline chemical family (Koo et al. 2013). It was originally developed for control of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] in rice (Hwang et al. 2005). Methiozolin is used in South Korea and is under development in Japan, Australia, and the United States for PRE and POST control of AB in BG turf, including putting greens. PRE and POST methiozolin applications can effectively control AB in CRBG turf, with little or no bentgrass injury (Brosnan et al. 2013; Flessner et al. 2013; Haguewood et al. 2012; Han and Kaminski 2012; Hart 2012; Hoyle et al. 2012; McCullough and Gòmez de Barrea 2012; Trappe et al. 2012).

Methiozolin uptake is through foliage and roots with moderate acropetal translocation in both AB and CRBG (Brosnan et al. 2013; Flessner et al. 2013; McCullough and Gòmez de Barrea 2012). Combined foliar and root applications or root applications alone have more success in controlling AB than foliar applications alone (Brosnan et al. 2013; Flessner et al. 2013). Methiozolin currently has two proposed modes of action. The first, less-studied mechanism suggests that methiozolin inhibits cell wall biosynthesis by a mechanism different from other herbicides that inhibit this process (Flessner et al. 2013; Hwang et al. 2005; Lee et al 2007; Nam et al. 2012). Whether this is a primary or secondary effect of methiozolin remains unknown (Flessner et al. 2013; Hwang et al. 2005; Lee et al 2007; Nam et al. 2012). The second mode of action proposed for methiozolin is inhibition of tyrosine aminotransferase (TAT) (Grossman et al. 2011). Inhibition of TATs in the plastoquinone and tocopherols synthesis pathway would have significant downstream effects; loss of plastoquinone function could ultimately lead to photo-oxidation and degradation of chlorophyll in growing tissues (Grossman et al. 2011).

Field studies have demonstrated that a single application of methiozolin is less effective in controlling AB in CRBG than sequential applications (Brosnan et al. 2013; Han and Kaminski 2012; Hoyle et al. 2012). Sequential applications of methiozolin at 2-wk intervals totaling 3.0 to 3.36 kg ha^{-1} were the most successful in controlling AB in putting green studies (Brosnan et al. 2013; Han and Kaminski 2012; McCullough et al. 2012). Field experiments previously conducted by the University of California, Riverside (UCR) suggest there might be differential tolerance to methiozolin among BG species and cultivars (J. H. Baird, unpublished data, 2011). To our knowledge no research has been published on differential bentgrass species or CRGB cultivar response to methiozolin; however, several studies documented differences in BG tolerance to other herbicides. Tolerance to herbicides such as fenoxaprop, metamifop, and bispyrabac-sodium varied among bentgrass species (Henry and Hart 2004; Kaminski 2005; McDonald et al. 2006; Straw et al. 2012). Tolerance of CRBG to bispyribac-sodium was greater than that of COBG and VBG (Kaminski 2005; McDonald et al. 2006). Certain CRBG cultivars were much more tolerant to metamifop than VBG (Straw et al. 2012). In contrast, VBG tolerated higher rates of fenoxaprop than CRBG (Henry and Hart 2004).

The objectives of this research were to examine differences in tolerance to methiozolin among CRBG, COBG, and VBG. Moreover, nine CRBG cultivars were tested to determine if herbicide tolerance exists among genotypes.

Materials and Methods

Research was conducted in greenhouses at UCR and at Auburn University (AU), in Auburn, AL. The AU study was conducted from February 16 to May 31, 2012. Average daily temperatures were not recorded, but greenhouse temperatures were modulated between 18 C and 23 C. Relative humidity averaged 68%. Plants received natural daylight only and photoperiods were 11 h and 14 h for February and May, respectively. The UCR study was conducted from May 29 to September 11, 2012. Average day and night greenhouse temperatures for the study were 28 C and 22 C, respectively, and relative humidity averaged 53%. Plants received natural daylight only and photoperiods were approximately 14 h and 12.5 h for May and September, respectively.

Nine cultivars of CRBG were chosen (Table 1) based upon several criteria, including widespread use, breeding background (i.e., genetic diversity), and perceived tolerance or sensitivity to methiozolin in the field. Velvet bentgrass and COBG cultivars chosen were 'SR 7200' and 'SR 7150', respectively (Table 1).

Both locations utilized 10.5 by 10.5 by 10.2-cm flats for the study. Rootzone mix used at AU was a 90 : 10 v/v (sand : sphagnum peat), pH 5.3. At UCR, the mix was an 85 : 15 v/v (sand : sphagnum peat), pH 6.8 (G-15S, P.W. Gillibrand Co., Inc., 4537 Ish Dr., Simi Valley, CA 93063). Bentgrasses were seeded at 48 kg ha^{-1} in both locations and allowed to establish for 7 wk. Overhead irrigation was applied two to three times daily to maintain adequate turfgrass quality. During establishment, plants were fertilized every 2 wk (28–8–16, 5.8 kg N ha^{-1} Miracle-Gro Water Soluble All Purpose Plant Food; The Scotts Company LLC, 14111 Scottslawn Road, Marysville, OH, 43040). Grasses were maintained at a height of 1.3 cm using scissors prior to initial treatment application.

At both locations, methiozolin was applied at 0, 0.6, 1.1, 2.2, 4.5, and 9.0 kg ha^{-1} at 7 and 12 wk after seeding. Treatments at AU were applied using an enclosed research spray cabinet with an 8006VS nozzle (Spraying Systems Co., P.O. Box 7900, Wheaton, IL, 60189). At UCR, treatments were applied using a four-nozzle CO_2-powered backpack sprayer (8004VS, Spraying Systems Co.). Both spraying systems were set to deliver 1,222 L ha^{-1}. Although the suggested application interval for methiozolin is every 2 wk, a 5-wk interval was utilized in these experiments to provide sufficient time to assess turf response following each application.

Turfgrass injury and clipping weights were determined 14 and 28 d after initial treatment (DAIT) and 7 and 21 d after second treatment (DAT2). Injury was visually rated on a scale of 0 to 100%, where 0% = no turfgrass injury visible and 100% = complete necrosis of all plants in the flat. Grasses were maintained at 1.3 cm, and clippings collected every 2 wk starting 14 DAIT. Dry weights were determined after drying clippings in a forced-air oven at 80 C for 72 h. Clipping dry weight (DW) as a percent of the nontreated control was calculated using Equation 1, allowing direct comparison of relative growth among bentgrass species and cultivars.

$$\begin{aligned} &\textit{Percent DW reduction} \\ &= [1 - (\textit{DW nontreated} - \textit{DW treated}) \\ &/(\textit{DW nontreated})]*100 \qquad (1) \end{aligned}$$

The experimental design at both locations was completely randomized with four replications in individual flats when comparing all individual cultivars. When comparing BG as a species, the nine CRBG cultivars were averaged into four replicates to directly compare CRBG to COBG and VBG species. Scatter plots of injury vs. methiozolin rate and percent DW of mean control vs. methiozolin rate suggested nonlinear relationships between respective variables. A four-parameter logistic curve, as described by Seefeldt et al. (1995), best describes nonlinear herbicidal dose response relationships. SigmaPlot software (version 12.3; Systat Software, Inc., 225 W Washington St., Suite 425, Chicago, IL 60606) was used to conduct regression analyses fitting injury and percent DW of mean control data to a four-parameter logistic curve. The methiozolin rate that caused 25% turfgrass injury (TI_{25}) and that caused a 50% reduction in clipping DW as a percentage of the nontreated control (GR_{50}) was calculated for each cultivar. The TI_{25} level was used because it represented minimally acceptable turfgrass quality and plant health. Fifty percent growth reduction in DWPC is the most widely used value in four-parameter logistic regression analyses for comparing plant sensitivities to herbicides, and thus was chosen for comparison (Seefeldt et al. 1995). Methiozolin rates that resulted in TI_{25} and GR_{50} values were subjected to ANOVA using Statistix 8 software (version 8, Analytical Software, PO Box 12185 Tallahassee, FL, 32317) followed by Fisher's

Table 1. Bentgrass species and cultivars used in the study, and clipping dry weight (g) of the nontreated controls collected at Auburn University (AU) and at University of California Riverside (UCR) 28 after initial treatment (DAIT) and 21 d after second treatment (DAT2).

			28 DAIT		21 DAT2	
Bentgrass species	Cultivar	Source	AU	UCR	AU	UCR
Creeping bentgrass	Focus	Seed Research of Oregon, Corvallis, OR	0.65	1.03	0.40	0.66
	T-1	J. R. Simplot Co., Boise, ID	0.63	1.30	0.59	0.81
	Penn G-2	Tee 2 Green, Hubbard, OR	0.27	1.39	0.34	0.90
	Bengal	Barenbrug USA, Tangent, OR	0.58	1.03	0.43	0.71
	007	Seed Research of Oregon	0.39	1.31	0.32	0.80
	Tyee	Seed Research of Oregon	0.31	0.76	0.29	0.30
	Penn A-4	Tee 2 Green	0.39	1.00	0.36	0.71
	96-2	Pickseed International, Lindsay, Ontario, Canada	0.27	0.83	0.19	0.65
	Penncross	Tee 2 Green	0.41	1.42	0.28	1.02
Colonial bentgrass	SR7150	Seed Research of Oregon	0.27	0.83	0.17	0.79
Velvet bentgrass	SR7200	Seed Research of Oregon	0.33	0.97	0.22	0.81

protected LSD test at the 0.05 probability level when appropriate.

Results and Discussion

Turf injury and growth reduction at 14 DAIT and 7 DAT2 were insufficient to allow logistic regression analyses. Lack of a significant herbicidal response was likely caused by the proximity of treatment application to sample collection, combined with slow symptom development following methiozolin treatment. Therefore, only data collected 28 DAIT and 21 DAT2 are presented.

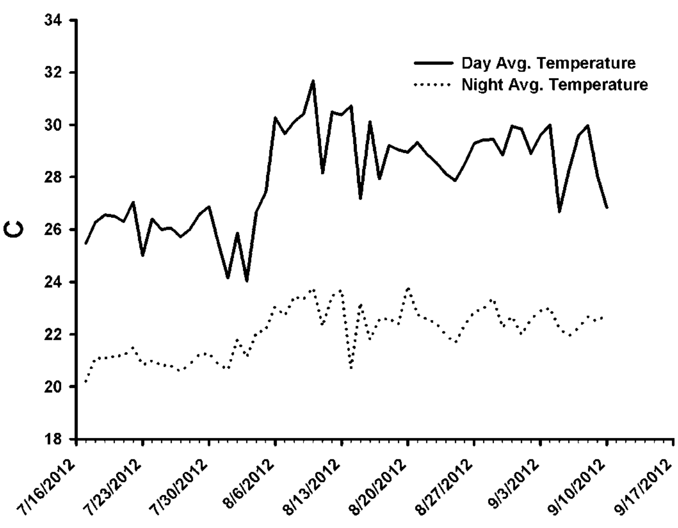

Figure 1. Average day and night temperature inside the greenhouse at University of California, Riverside (UCR). Day temperatures represent an average of temperatures collected every 5 min from 7 A.M. to 7 P.M. Night temperatures represent an average of temperatures collected every 5 min from 7 P.M. to 7 A.M.

Average day and night greenhouse temperatures during the study at UCR are shown in Figure 1. Although similar data were not collected at AU, greenhouse temperatures were considerably lower than UCR because the AU study was conducted during late winter and spring. Greenhouse temperatures were also modulated at UCR; however, higher outside temperatures appeared to raise temperatures inside the greenhouse. Average monthly outside air temperatures in Riverside ranged from 19 to 27 C during the study period and the average maximum outside air temperature was 35 C in August (data not shown). Clipping dry weights collected 28 and DAIT and 21 DAT2 from nontreated plants are listed in Table 1. In general, all species and cultivars responded to higher temperatures at UCR with increased growth except for CRBG 'Tyee', which grew similarly at both locations 21 DAT2.

Differences Among Bentgrass Species. Four-parameter logistic regression closely estimated species injury and growth reduction caused by methiozolin at 28 DAIT and 21 DAT2. For percent DW of mean control, regression coefficients (R^2) ranged from 0.83 to 0.99 28 DAIT and 0.84 to 0.99 21 DAT2 (Figures 2 and 3). Regression coefficients for injury ranged from 0.95 to 0.99 28 DAIT and 0.89 to 0.99 56 DAIT (Figures 4 and 5). A period of "post inhibition growth enhancement," similar to effects of growth regulators such as trinexapac-ethyl and paclobutrazol, was observed for

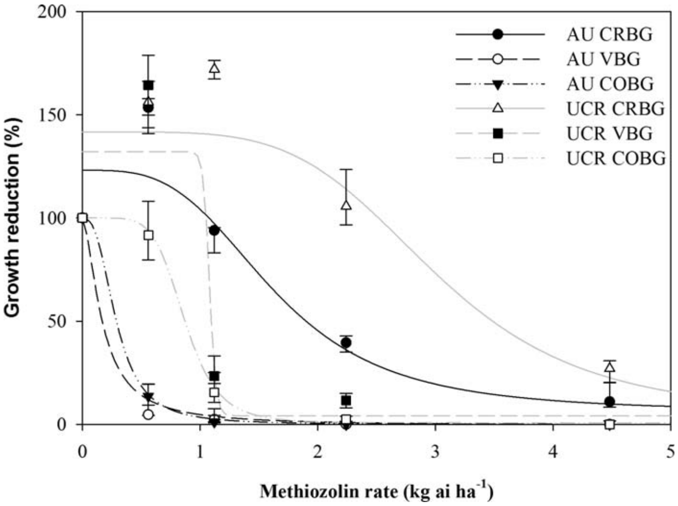

Figure 2. Percent growth reduction of creeping bentgrass (CRBG), velvet bentgrass (VBG), and colonial bentgrass (COBG) treated with methiozolin at 0, 0.6, 1.1, 2.2, 4.5, or 9.0 kg ai ha^{-1} 7 wk after seeding (0 d after initial treatment, DAIT). Data were collected 28 DAIT at Auburn University (AU) and University of California, Riverside (UCR). Data points represent an average of four replications. Error bars indicate standard errors. A four-parameter logistic regression model of $y = y_0 + a/(1+(x/x_0)^b)$ was used to fit data. Regression parameters and coefficients of determination at AU were CRBG a = 117.1, b = 3.4, $x_0 = 1.6$, $y_0 = 6.1$, $R^2 = 0.88$; COBG a = 99.7, b = 2.7, $x_0 = 0.3$, $y_0 = 0.0$, $R^2 = 0.99$; VBG a = 100.6, b = 1.6, $x_0 = 0.2$, $y_0 = -0.6$, $R^2 = 0.98$. Regression parameters and coefficients of determination at UCR were CRBG a = 137.4, b = 4.68, $x_0 = 3.0$, $y_0 = 4.3$, $R^2 = 0.83$; COBG a = 99.2, b = 6.2, $x_0 = -0.9$, $y_0 = 0.8$, $R^2 = 0.94$; VBG a = 128.0, b = 41.7, $x_0 = 1.1$, $y_0 = 4.2$, $R^2 = 0.87$.

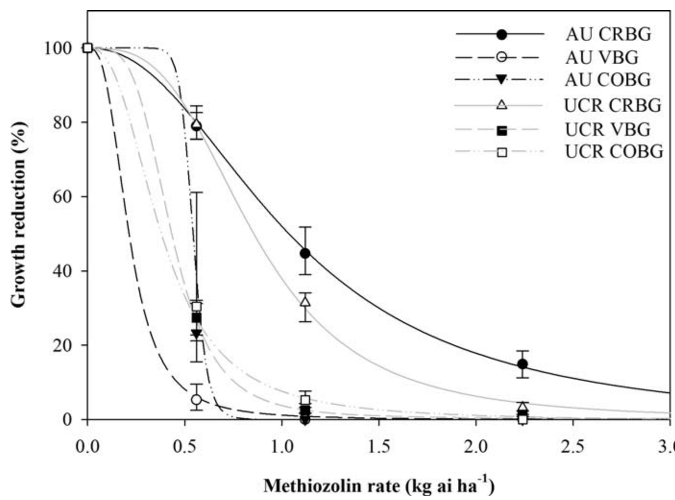

Figure 3. Percent growth reduction of creeping bentgrass (CRBG), velvet bentgrass (VBG), and colonial bentgrass (COBG) treated with either methiozolin at 0, 0.6, 1.1, 2.2, 4.5, or 9.0 kg ai ha^{-1} 7 wk after seeding (0 d after initial treatment, DAIT), with a second application at 35 DAIT. Data were collected 56 DAIT at Auburn University (AU) and University of California, Riverside (UCR). Data points represent an average of four replications. Error bars indicate standard errors. A four-parameter logistic regression model of $y = y_0 + a/(1+(x/x_0)^b)$ was used to fit data. Regression parameters and coefficients of determination at AU were CRBG a = 101.9, b = 2.2, $x_0 = -1.1$, $y_0 = -1.9$, $R^2 = 0.97$; COBG a = 100.0, b = 15.3, $x_0 = 0.5$, $y_0 = 0.0$, $R^2 = 0.84$; VBG a = 100.0, b = 2.8, $x_0 = -0.2$, $y_0 = 0.0$, $R^2 = 0.99$. Regression parameters and coefficients of determination at UCR were CRBG a = 100.0, b = 3.2, $x_0 = 0.9$, $y_0 = -0.1$, $R^2 = 0.99$; COBG a = 100.3, b = 2.5, $x_0 = -0.4$, $y_0 = -0.3$, $R^2 = 0.99$; VBG a = 99.7, b = 4.0, $x_0 = -0.4$, $y_0 = 0.3$, $R^2 = 0.99$.

all CRBG cultivars at 28 DAIT at both locations, and will be hereafter referred to as the "rebound effect" (Branham and Beasley 2007; Ervin and Zhang 2008; Fagerness and Yelverton 2000; Kreuser and Soldat 2011; McElroy et al. 2013). Creeping bentgrass, averaged across nine cultivars at the 0.56 kg ha^{-1} rate, had a mean growth increase of 153% compared to nontreated plants at AU (Figure 2); the rebound effect was not observed at higher rates or for COBG or VBG at AU. At UCR, CRBG exhibited a rebound effect at the 0.56, 1.12 and 2.24 kg ha^{-1} methiozolin rates with respective increases in growth of 156, 172, and 106%; unlike AU, VBG treated with 0.56 kg ha^{-1} methiozolin showed a 164% increase in growth at UCR (Figure 2). However, similar to AU, the rebound effect was not observed for COBG at UCR (Figure 2). Differences between locations in rebound effects among turfgrass species following methiozolin application might have been due to greenhouse air temperatures. There was no rebound effect observed at 21 DAT2 at either location; this could be attributed to the proximity of the collection date to the second application (Figure 3), or to greater toxicity following the second application that prevented the rebound from happening.

Analysis of GR_{50} at both 28 DAIT and 21 DAT2 revealed a significant two-way interaction between species and location at the 0.05 probability level. Consequently, GR_{50} data were subjected to Fisher's protected LSD and are presented separately for each combination of grass species and location (Table 2). At 28 DAIT at AU, averaged over cultivar, CRBG had a GR_{50} of 1.9 kg ha^{-1} following methiozolin application compared to 0.4 kg ha^{-1} for COBG and VBG (Table 2). Methiozolin rates required to achieve GR_{50} 28 DAIT were higher for each grass species at UCR than at AU (Table 2); these results might be due to the fact that higher clipping dry

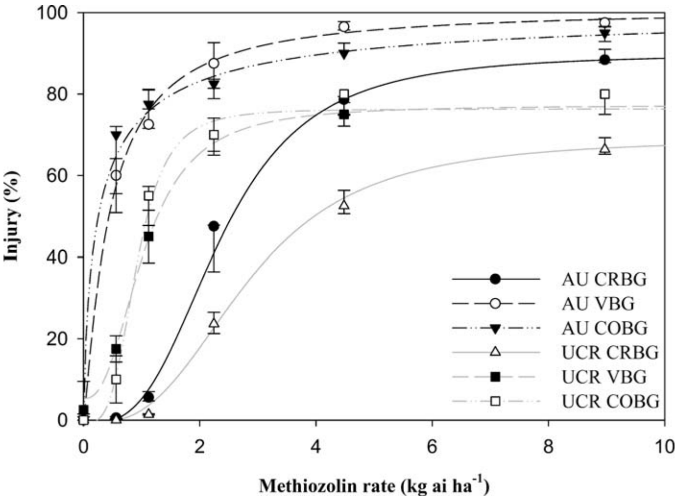

Figure 4. Percent injury of creeping bentgrass (CRBG), velvet bentgrass (VBG), and colonial bentgrass (COBG) treated with methiozolin at 0, 0.6, 1.1, 2.2, 4.5, or 9.0 kg ai ha^{-1} 7 wk after seeding (0 d after initial treatment, DAIT). Data were collected at 28 DAIT at Auburn University (AU) and University of California, Riverside (UCR). Data points represent an average of four replications. Error bars indicate standard errors. A four-parameter logistic regression model of $y = y_0 + a / (1+(x/x_0)^b)$ was used to fit data. Regression parameters and coefficients of determination at AU were CRBG a = 90.7, b = −3.1, x_0 = 2.4, y_0 = −0.8, R^2 = 0.98; COBG a = 101.2, b = −0.7, x_0 = 0.3, y_0 = 0.0, R^2 = 0.96; VBG a = 101.8, b = −1.2, x_0 = 0.4, y_0 = 0.0, R^2 = 0.96. Regression parameters and coefficients of determination at UCR were CRBG a = 69.8, b = −2.9, x_0 = 2.8, y_0 = −0.3, R^2 = 0.98; COBG a = 76.9, b = −3.6, x_0 = −0.9, y_0 = −0.5, R^2 = 0.96; VBG a = 71.94, b = −2.5, x_0 = −1.0, y_0 = 5.3, R^2 = 0.94.

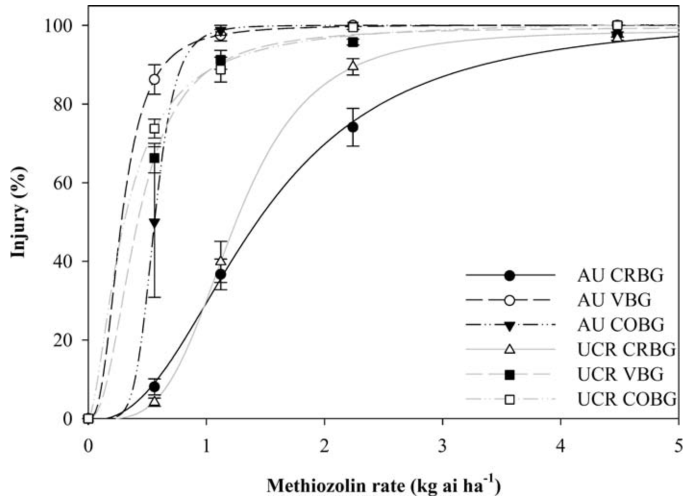

Figure 5. Percent injury of creeping bentgrass (CRBG), velvet bentgrass (VBG), and colonial bentgrass (COBG) treated with methiozolin at 0, 0.6, 1.1, 2.2, 4.5, or 9.0 kg ai ha^{-1} 7 wk after seeding (0 d after initial treatment, DAIT), with a second application at 35 DAIT. Data were collected 56 DAIT at Auburn University (AU) and University of California, Riverside (UCR). Data points represent an average of four replications. Error bars indicate standard errors. A four-parameter logistic regression model of $y = y_0 + a/(1+(x/x_0)^b)$ was used to fit data. Regression parameters and coefficients of determination at AU were CRBG a = 102.7, b = −2.34, x_0 = 1.4, y_0 = −0.5, R^2 = 0.99; COBG a = 100.0, b = −6.3, x_0 = 0.6, y_0 = 0.0, R^2 = 0.89; VBG a = 100.1, b = −2.6, x_0 = 0.3, y_0 = 0.0, R^2 = 0.99. Regression parameters and coefficients of determination at UCR were CRBG a = 98.9, b = −3.8, x_0 = 1.2, y_0 = −0.2, R^2 = 0.99; COBG a = 100.4, b = −1.6, x_0 = −0.3, y_0 = 0.0, R^2 = 0.99; VBG a = 99.6, b = −2.3, x_0 = 0.4, y_0 = 0.0, R^2 = 0.99.

weights were collected at UCR in comparison to AU (Table 1). At UCR, the rates were 3.2, 0.9 and 1.1 kg ha^{-1} for CRBG, COBG, and VBG, respectively. Despite the difference in the magnitude of the rate for GR_{50} between locations, similar trends were observed among grass species; the methiozolin rate for GR_{50} of CRBG was significantly greater than that of COBG and VBG at both locations. In contrast, 21 DAT2, a higher methiozolin rate was required for CRBG GR_{50} at AU (1.1 kg ha^{-1}) than at UCR (0.8 kg ha^{-1}) (Table 2). Differences in methiozolin rate for GR_{50} between COBG (0.6 kg ha^{-1}) and VBG (0.3 kg ha^{-1}) were detected at AU only; GR_{50} was achieved at 0.4 kg ha^{-1} for both species at UCR (Table 2).

Analysis of variance for TI_{25} 28 DAIT revealed significant species and location effects at the 0.05 probability level; therefore, data were initially pooled over location and presented separately for grass species, and subsequently pooled over grass species and presented separately for each location. Creeping bentgrass showed greater tolerance to methiozolin compared to the other species, with 25% of turfgrass injury occurring at a rate of 2.4 kg ha^{-1} (Table 2). When data were pooled across grass species, the TI_{25} values were 1.5 kg ha^{-1} and 0.8 kg ha^{-1} at UCR and AU, respectively (data not shown).

Only species effects were observed 21 DAT2 for TI_{25} ($P = 0.05$); therefore, data were pooled across location and presented separately for grass species. Creeping bentgrass was the least injured from methiozolin application; mean TI_{25} observed for CRBG resulted from 1.1 kg ha^{-1} when averaged across locations (Table 2). In contrast, COBG and VBG required only 0.3 and 0.2 kg ha^{-1} methiozolin, respectively, to cause 25% injury (Table 2).

Methiozolin rates that caused 25% injury (Table 2) were lower than methiozolin rates that caused 50% reduction in growth for respective species and rating dates, with the exception of CRBG at AU 28

Table 2. Methiozolin rate (kg ai ha^{-1}) that caused a 50% growth reduction (GR_{50}) and 25% turfgrass injury (TI_{25}) in creeping, colonial, and velvet bentgrass at Auburn University (AU) and at University of California Riverside (UCR) 28 d after initial treatment (DAIT) and 21 d after second treatment (DAT2). Methiozolin treatment was initiated 7 wk after seeding (0 DAIT), with a second application 35 DAIT. Values for TI_{25} were pooled over two locations; Auburn University (AU) and University of California Riverside (UCR).

Bentgrass species[a]	Location	GR_{50} and TI_{25}			
		28 DAIT		21 DAT2	
		kg ai ha^{-1} methiozolin			
Creeping	AU	1.9	b[b]	1.1	a[b]
Colonial		0.4	d	0.6	bc
Velvet		0.4	d	0.3	d
Creeping	UCR	3.2	a	0.8	b
Colonial		0.9	c	0.4	cd
Velvet		1.1	c	0.4	cd
Creeping	AU/UCR	2.4	a	1.1	a
Colonial		0.5	b	0.3	b
Velvet		0.5	b	0.2	b

[a] For bentgrass species, "creeping" denotes pooled data from nine creeping bentgrass cultivars, and "colonial" and "velvet" denote data from one cultivar.

[b] Means separated by LSD followed by the same letter in a column are not significantly different ($\alpha = 0.05$).

DAIT (Table 2). These results suggest that methiozolin rates that cause 50% reduction in growth would cause unacceptable injury levels for turfgrass; consequently, in this study TI_{25} rates were considered more critical for assessing species tolerance. However, after one application of methiozolin, rates that caused TI_{25} for COBG and VBG were equal to the recommended rate for methiozolin on putting greens (0.5 kg ha^{-1}; SJ Koo, personal communication, Moghu Research Center). Control of AB requires multiple sequential applications of methiozolin (Brosnan et al. 2013; Han and Kaminski 2012; McCullough et al. 2012). Therefore, TI_{25} rates 21 DAT2 (two applications) are thought to be the most accurate indicator of species tolerance in our study. Results from this study suggest that CRBG is tolerant to two methiozolin applications, whereas COBG and VBG species are sensitive to sequential methiozolin applications at rates that would control AB. Significant turf injury occurred after only two applications of methiozolin in the greenhouse, given the herbicide rates and experimental units (pots) used in this study. Therefore, additional applications were not made.

Differences among Creeping Bentgrass Cultivars. Four-parameter logistic regression analysis, for both percent DW of mean control and injury data, was performed for each of nine CRBG cultivars examined in this study. Regression analysis fit closely to both percent DW of mean control and injury data; regression coefficients for percent DW of mean control ranged from 0.57 to 1.0 at 28 DAIT and from 0.61 to 1.0 21 DAT2; regression coefficients for injury data ranged from 0.92 to 1.0 28 DAIT and from 0.84 to 1.0 21 DAT2. Due to the large number of cultivars evaluated, graphical representation of the regressions is not shown.

Analysis of variance of CRBG cultivar GR_{50} data revealed no significant effects or interactions among CRBG cultivars and location 28 DAIT; however, a cultivar by location interaction was detected 21 DAT2 ($P = 0.05$). Rates of methiozolin resulting in GR_{50} ranged from 0.5 ('Focus' and '007') to 2.6 kg ha^{-1} (Penn A-4) at AU and 0.7 ('T-1') to 1.0 kg ha^{-1} (Bengal) at UCR 21 DAT2 (Table 3). Creeping bentgrass Penn A-4 demonstrated the greatest tolerance to methiozolin at AU. This cultivar also required the highest rate for GR_{50} at UCR, but the rate was not significantly different from other cultivars (Table 3). The lack of significance among bentgrass cultivars at UCR might be due to high day average temperature occurring in the greenhouse 21 DAIT. Optimal temperatures for creeping bentgrass photosynthesis range from 15 to 24 C (Beard, 1973). Starting from the first week of August, greenhouse temperatures during the day were constantly supraoptimal for creeping bentgrass growth at UCR; in fact, clipping dry weights

Table 3. Methiozolin rate that caused a 50% growth reduction (GR_{50}) in eight different creeping bentgrass cultivars at Auburn University (AU) and at University of California Riverside (UCR) 21 d after second treatment (DAT2), and the rate that caused 25% Turfgrass Injury (TI_{25}) in eight different creeping bentgrass cultivars at AU and UCR 28 d after initial treatment (DAIT and 21 d after second treatment (DAT2). Methiozolin treatment was initiated 7 wk after seeding (0 DAIT) with a second application 35 DAIT.

Location	Cultivar	GR_{50}	TI_{25}	
			28 DAIT	21 DAT2
		kg ai ha^{-1} methiozolin		
AU	Focus	0.5 c[a]	2.4 cdef[a]	0.7 d[a]
	T-1	0.8 bc	2.2 cdef	0.9 d
	Penn G-2	1.0 bc	1.8 efg	1.4 bc
	Bengal	1.4 b	1.9 efg	1.5 b
	007	0.5 c	2.3 cdef	0.6 d
	Tyee	0.7 c	2.0 defg	0.8 cd
	Penn A-4	2.6 a	2.8 cd	2.5 a
	96-2	1.0 bc	2.1 defg	1.0 bcd
	Penncross	0.9 bc	1.3 g	1.0 bcd
UCR	Focus	0.8 c	2.7 cde	1.0 bcd
	T-1	0.7 c	2.5 cdef	0.9 bcd
	Penn G-2	0.8 bc	2.6 cdef	0.9 bcd
	Bengal	1.0 bc	3.8 ab	1.1 bcd
	007	0.9 bc	2.1 defg	1.0 bcd
	Tyee	0.8 bc	2.0 defg	0.9 bcd
	Penn A-4	0.9 bc	4.5 a	0.8 bcd
	96-2	0.8 c	3.1 bc	1.0 bcd
	Penncross	0.8 bc	2.0 defg	1.1 bcd

[a] Means separated by LSD followed by the same letter in a column are not significantly different ($\alpha = 0.05$).

collected 21 DAT2 from the nontreated controls were lower than those collected 28 DAIT. Therefore, heat stress might have diminished tolerance to the second methiozolin application at UCR.

Analysis of variance for mean TI_{25} rates indicated location by cultivar interactions for both 28 DAIT and 21 DAT2 at the 0.05 probability level. Methiozolin rates that caused TI_{25} 28 DAIT ranged from 1.3 ('Penncross') to 4.5 kg ha^{-1} (Penn-A-4; Table 3). Penn A-4 tolerance to methiozolin was already documented by McNulty et al. (2011) who reported no injury when methiozolin was applied up to 4 kg ha^{-1}. Fifty-six DAIT, methiozolin rate for TI_{25} ranged from 0.6 to 2.5 kg ha^{-1} (Table 3). Although no differences were observed among cultivars at UCR, Penn A-4 was the cultivar that tolerated the highest methiozolin rate at AU (Table 3). Once again, high temperatures in the greenhouse might be the plausible cause for lack of consistency in results at UCR; in fact, heat stress might have enhanced turf injury, causing TI_{25} rates to be lower than those recorded at AU. Even though results were inconsistent between locations, the CRBG cultivar Penn A-4 exhibited the highest herbicide tolerance overall at both sites. Similar to the species effect, there was a decrease in magnitude of the methiozolin rate for TI_{25} among cultivars between 28 DAIT and 21 DAT2 (Table 3). From these studies, CRBG appears to be more tolerant than COBG and VBG to one or two applications of methiozolin. For CRBG cultivars tested in this study, GR_{50} and TI_{25} occurred only at methiozolin rates above the recommended rate for putting greens. At both AU and UCR, Penn A-4 exhibited the least growth regulation and injury in response to methiozolin application when significant effects among CRBG cultivars were apparent. COBG and VBG are unlikely to tolerate sequential applications of methiozolin at a rate that is adequate to effectively control AB. Further research is needed to accurately describe the relative tolerances among creeping bentgrass cultivars under field conditions, and to determine if temperature is a key factor in methiozolin tolerance.

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